INSTITUUT VOOR PLANTENZIEKTENKUNDIG ONDERZOEK
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HET TOETSEN VAN AARDAPPELKNOLLEN OP DE AANWEZIGHEID VAN Y^N-VIRUS

(TESTING OF POTATO TUBERS FOR THE PRESENCE OF VIRUS Y^N)

DOOR

J. A. DE BOKX

POTATO VIRUSES AND SOME REMARKS ON THEIR CONTROL

DOOR

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HET TOETSEN VAN AARDAPPELKNOLLEN OP DE AANWEZIGHEID VAN Y^N-VIRUS[1]

With a summary: Testing of potato tubers for the presence of virus Y^N

DOOR

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INLEIDING

Als het gebruik van aangesneden aardappelknollen als inoculum voor de bladtoets een betrouwbaar resultaat zou geven omtrent de aanwezigheid van virus in de knol, zou de toetsing van pootgoed veel sneller kunnen geschieden dan indien sap van spruiten of bladeren als inoculum aangewend moet worden. NIENHAUS (1960) voerde een aantal experimenten uit met tabak als toetsplant en aangesneden, kunstmatig tot spruiten gebrachte knollen als inoculum. De knollen, die secundair met Y-virus waren besmet, werden aan het naveleinde aangesneden. De verkregen snijvlakken werden vervolgens met de toetsbladeren in aanraking gebracht. Volgens deze methode zou het Y-virus zeer betrouwbaar kunnen worden aangetoond.

Het zou echter nog belangrijker zijn, indien vroeg gerooide, niet spruitende knollen volgens deze methode getoetst zouden kunnen worden. In het volgende zullen daarom een aantal proeven worden beschreven, die werden uitgevoerd met het doel gegevens te verkrijgen omtrent de mogelijkheid van het toetsen van knollen op verschillende tijdstippen na het rooien.

MATERIAAL EN METHODIEK

Door aardappelplanten in de kas met Y^N-virus te inoculeren, werden in het najaar van 1960 knollen verkregen, die in meer of mindere mate met dit virus besmet waren. Van deze knollen werd met een ontsmet mes een kapje van ongeveer $^1/_2$ cm dikte, zowel van het basale als van het apicale deel van de knol, afgesneden. De aldus aan de knol verkregen snijvlakken werden ieder op een indicatorblad getoetst. Deze toetsing, aangeduid met „knoltoetsing", geschiedde door het snijvlak van de knol onder zachte druk in aanraking te brengen met de toetsbladeren, die van te voren lichtelijk bestrooid waren met carborundumpoeder 500 mesh. Verder werden knollen op aanwezigheid van Y^N-virus onderzocht met behulp van spruitinoculum (= „spruittoetsing"). Hierbij werden spruiten met behulp van een handpers gekneusd. De gekneusde spruitenmassa werd vervolgens met een ontsmet pincet met de toetsbladeren in aanraking gebracht. De ontsmetting van mes en pincet geschiedde door dompeling in een verzadigde zeepoplossing, gevolgd door afspoeling met stromend leidingwater.

Veelal verkeerden de knollen nog in kiemrust omdat direkt na het rooien met

[1] Aangenomen voor publikatie 25 aug. 1961.

de toetsingen werd aangevangen. Om echter snel over aardappelspruiten te kunnen beschikken, werd de kiemrust der knollen kunstmatig met behulp van „rindite" gebroken (DENNY, 1945). Nadat de knollen en spruiten op aanwezigheid van Y-virus waren onderzocht, werden de knollen in de kas uitgeplant. Het bladsap van de uit deze knollen gegroeide plantjes werd vier weken na het planten met behulp van de bladtoets op aanwezigheid van Y-virus onderzocht. Deze laatste toetsing, in het vervolg aangeduid met „plantjestoetsing", werd bepalend geacht voor het al of niet aanwezig zijn van het virus in de knol.

Door van kunstmatig geïnfecteerde planten uit te gaan, kan worden nagegaan welk verband er bestaat tussen de leeftijd der geïnoculeerde planten, de aanwezigheid van virus in de knol en de resultaten van de toetsingen. Verder behoeft er geen twijfel te bestaan aangaande het virus, dat wordt getoetst. Bij knollen, verzameld in praktijkvelden, is het nl. mogelijk dat een ander aardappelvirus in het spel is, dat geen of onduidelijke symptomen op het toetsblad doet ontstaan.

Als toetsplant werd vrijwel alleen „A6" (kruising van *Solanum demissum* en Aquila) gebruikt. De toetsingen werden hoofdzakelijk in de wintermaanden uitgevoerd. Hoewel de toegepaste bijbelichting bij de kruising A6 niet tot een maximale bladproduktie leidde, was deze onder de genoemde omstandigheden toch veel beter dan bij *Solanum demissum* „Y" (SdY). In vele gevallen ging *Solanum demissum* „Y" onder de gebruikte lage lichtintensiteit nl. tot knolvorming over en stierf vervolgens af.

Voor elke toetsing werd slechts één toetsblad gebruikt. De bewaring en behandeling der toetsbladeren werd op reeds eerder beschreven wijze uitgevoerd (DE BOKX, 1960).

RESULTATEN

De eerste proef bestond uit drie series planten van het ras Bintje, die respectievelijk op een leeftijd van zes, acht en tien weken besmet werden met Y^N-virus. In elk serie werden acht planten gerooid, respectievelijk 10, 17 en 24 dagen na inoculatie. Van elke plant kon slechts de grootste knol voor toetsing op A6-blad worden gebruikt, daar de resterende knollen voor het verkrijgen van gegevens betreffende ouderdomsresistentie benut moesten worden. De toetsing van de knollen geschiedde 4 weken na het rooien van de laatste serie, waaruit volgt, dat de knollen van de eerste serie toen al 10 weken waren bewaard. De toetsing der knollen had plaats voordat de kiemrust der knollen kunstmatig werd gebroken. In tabel 1 zijn de uitkomsten van de eerste proef weergegeven.

Een vrij hoog percentage der besmette knollen van de planten, die op een leeftijd van zes en acht weken waren geïnoculeerd, kon achterhaald worden. Het is opvallend, dat bij planten die werden geïnoculeerd toen zij 10 weken oud waren, slechts besmetting bij één knol werd aangetoond. Uit de gegevens zou geconcludeerd kunnen worden, dat met het krooneinde van de knol als inoculum in meer knollen het Y^N-virus kon worden vastgesteld dan met het naveleinde.

In deze proef zijn ook toetsingen op SdY-blad uitgevoerd. Uit tabel 2 blijkt, dat de resultaten hiervan niet overeenstemmen met die, verkregen met A6-toetsblad. Uit de gegevens blijkt nl., dat hier met aangesneden naveleinden

TABEL 1. Verband tussen het aantonen van Y^N-virus in de knol (ras Bintje) met A6-toetsblad en het tijdstip van inoculatie en rooien van de plant.
Relation between the detection of virus Y^N in tubers by means of A6-testleaves and the dates of inoculation and harvesting.

Leeftijd der planten in weken bij inoculatie *Age of plants in weeks at inoculation*	Aantal dagen tussen inoculatie en rooien *Interval in days between inoculation and harvesting*	Naveleinde[1] *Heel end of tuber*[1]	Krooneinde[1] *Eye end of tuber*[1]	Aantal knollen waarin aan navel- en kroon-einde virus werd aangetoond *Number of infected tubers in which virus was detected at both heel- and eye-ends*	Werkelijk aantal knollen met virus besmet (volgens plantjestoetsing) *Number of tubers infected by the virus detected by means of "tuber indexing"*
6	10	4/8	3/8	2	8
	17	3/6	5/6	2	6
	24	6/8	7/8	5	8
8	10	3/7	1/7	1	7
	17	1/8	4/8	0	7
	24	2/8	7/8	1	8
10	10	0/8	0/8	0	8
	17	1/8	0/8	0	8
	24	0/8	0/8	0	8

[1] Teller = aantal knollen, waarin Y^N-virus werd aangetoond.
Noemer = totaal aantal knollen.
[1] *Numerator = number of infected tubers detected by test.*
Denominator = total number of tubers tested.

TABEL 2. Verband tussen het aantonen van Y^N-virus in de knol (ras Bintje) met SdY-toetsblad en het tijdstip van inoculatie en rooien van de plant.
Relation between the detection of virus Y^N in the tubers by means of SdY-test leaves and the dates of inoculation and harvesting.

Leeftijd der planten in weken bij inoculatie *Age of plants in weeks at inoculation*	Aantal dagen tussen inoculatie en rooien *Interval in days between inoculation and harvesting*	Naveleinde[1] *Heel end*[1]	Krooneinde[1] *Eye end*[1]	Werkelijk aantal knollen met virus besmet (volgens plantjestoetsing) *Number of tubers infected by the virus detected by means of "tuber indexing"*	Aantal knollen waarin Y-virus werd vastgesteld door A6- en SdY-toetsblad *Number of infected tubers detected on both A6- and SdY-testleaves*	
					Naveleinde *Heel end*	Krooneinde *Eye end*
6	10	5/8	2/8	8	5	4
	17	4/6	1/6	6	6	5
	24	7/8	2/8	8	8	7
8	10	5/7	1/7	7	6	2
	17	2/8	3/8	7	2	5
	24	2/8	1/8	8	4	7
10	10	0/8	0/8	8	–	–
	17	0/8	0/8	8	–	–
	24	1/8	1/8	8	–	–

[1] Teller = aantal knollen, waarin Y^N-virus werd aangetoond.
Noemer = totaal aantal knollen.
[1] *Numerator = number of infected tubers detected by test.*
Denominator = total number of tubers tested.

als inoculum de meeste besmette knollen aangetoond kunnen worden. Het is niet te verklaren, waarom de resultaten met A6 en SdY niet met elkaar in overeenstemming zijn. Worden de resultaten van beide toetsplanten gecombineerd, dan is er geen verschil tussen de uitkomsten voor de beide einden van de knol. Hierbij moet wel bedacht worden, dat in de series met A6 en SdY niet steeds dezelfde knollen als ziek werden aangetoond. Geconcludeerd kan worden, dat meer met Y^N-virus besmette knollen konden worden aangetoond naarmate het tijdstip van rooien later werd gekozen.

Een tweede, soortgelijke proef werd uitgevoerd met planten van het ras Record. De inoculatie met Y^N-virus werd uitgevoerd op een blad halverwege de stengel, toen de planten zes weken oud waren. Het rooien van telkens vijf planten geschiedde respectievelijk 13, 19, 21, 26, 29 en 34 dagen na inoculatie. Bij deze proef werden vrijwel alleen de naveleinden van de knollen op aanwezigheid van Y^N-virus getoetst. De toetsing van de krooneinden werd slechts uitgevoerd bij rooien 29 dagen na inoculatie. In dit geval werd in dezelfde knollen virus aangetoond als bij toetsing van de naveleinden.

Uit tabel 3 blijkt, dat vlak na het rooien geen virus in de knol kon worden aangetoond, wel echter in knollen die vrij lang na de inoculatie werden gerooid.

TABEL 3. Aantal knollen (ras Record), waarin Y^N-virus werd aangetoond door middel van de A6-bladtoets op het tijdstip van rooien en vier weken na het rooien. Het aangesneden naveleinde van de knol is als inoculum gebruikt.
Number of infected tubers detected by means of the A6-leaf test when tested at harvest time and four weeks after harvest. The cut heel end of the tuber was used as inoculum.

Aantal dagen tussen inoculatie en rooien *Interval in days between inoculation and harvesting*	Getoëtst op tijdstip van rooien[1] *Tested at harvesting*[1]	Getoetst vier weken na rooien[1] *Tested four weeks after harvesting*[1]	Aantal besmette knollen volgens plantjestoetsing *Number of infected tubers detected by "tuber indexing"*
13	0/30 = 0%	17/38 = 56%	11/16 = 70%
19	0/45 = 0%	31/45 = 69%	36/39 = 92%
21	0/39 = 0%	17/39 = 43%	31/33 = 94%
26	7/43 = 16%	29/48 = 60%	22/25 = 88%
29	12/49 = 24%	48/49 = 98%	27/34 = 80%
34	17/43 = 40%	15/43 = 35%	26/29 = 90%

[1] Teller = aantal knollen, waarin Y^N-virus werd vastgesteld.
Noemer = totaal aantal knollen.
[1] *Numerator = number of infected tubers detected by test.*
Denominator = total number of tubers tested.

Het is niet uit te maken, op welk moment na de inoculatie alle knollen als ziek kunnen worden onderkend. De leeftijd der planten speelt hierbij waarschijnlijk een rol, zoals uit de resultaten van de derde proef blijkt. Deze proef bestond uit planten van het ras Bintje, die respectievelijk zeven, acht, negen en tien weken na het planten met Y^N-virus geïnoculeerd werden. Het rooien der acht en negen weken oude planten had respectievelijk zeven en 14 dagen na inoculatie plaats, terwijl de zeven en tien weken oude planten respectievelijk 42 en 14 dagen na inoculatie werden gerooid.

Volgens de resultaten van deze proef (tabel 4) kon bij planten van het ras Bintje, die op een leeftijd van zeven weken waren geïnoculeerd, 42 dagen na inoculatie met het Y^N-virus, bij gebruik van aangesneden knollen als inocu-

lum, in 76% van de besmette knollen het Y^N-virus direct bij het rooien worden aangetoond.

TABEL 4. Aantal knollen (ras Bintje), waarin Y^N-virus werd vastgesteld door middel van de A6-bladtoets onmiddellijk en zes weken na het rooien. Het aangesneden naveleinde der knol is als inoculum gebruikt.
Number of infected tubers detected by means of A6-leaf test when tested at harvest time and six weeks after harvest. The cut heel end of the tuber was used as inoculum.

Leeftijd der planten in weken bij inoculatie *Age of plants in weeks at inoculation*	Aantal dagen tussen inoculatie en rooien *Interval in days between inoculation and harvesting*	Getoetst op tijdstip van rooien[1] *Tested at time of harvesting*[1]	Getoetst zes weken na het rooien, twee weken na het breken van de kiemrust[1] *Tested six weeks after harvesting, two weeks after breaking dormancy*[1]	Aantal besmette knollen volgens plantjestoetsing[1] *Number of infected tubers detected by "tuber indexing"*[1]	Aantal besmette knollen volgens spruittoetsing[1] *Number of infected tubers detected by testing sprouts*[1]
7	42	22/29 = 76%	27/29 = 93%	29/29 = 100%	29/29 = 100%
8	7	2/14 = 14%	2/14 = 14%	2/14 = 14%	1/14 = 7%
	14	0/19 = 0%	15/19 = 80%	17/19 = 90%	17/19 = 90%
9	7	0/15 = 0%	10/15 = 66%	14/15 = 92%	14/15 = 92%
	14	0/10 = 0%	9/10 = 90%	10/10 = 100%	10/10 = 100%
10	14	0/24 = 0%	10/24 = 41%	22/24 = 91%	19/24 = 80%

[1] Teller = aantal knollen, waarin Y^N-virus werd aangetoond
Noemer = totaal aantal knollen.
[1] *Numerator = number of infected tubers detected by test.*
Denominator = total number of tubers tested.

Uit tabel 4 kan, evenals uit tabel 3, geconcludeerd worden dat direct bij het rooien – indien reeds gerooid wordt 14 dagen na inoculatie – meestal geen besmette knollen aangetoond kunnen worden door middel van de bladtoets. Een bewaring van zes weken, nl. vier weken vóór en twee weken na het breken van de kiemrust, heeft het percentage besmette knollen dat onderkend kan worden sterk doen stijgen. Zoals verder uit de tabel blijkt, geeft het gebruik van spruitinoculum in het algemeen betere resultaten dan dat van knolinoculum.

Om het gebruik van spruitinoculum nogmaals aan een onderzoek te onderwerpen, werd in oktober 1960 een vierde proef uitgevoerd, waarbij 27 planten van het ras Bintje 11 weken na het planten met Y^N-virus werden geïnoculeerd. Hiervan werden 13 planten 9 dagen en 14 planten 14 dagen na inoculatie gerooid. Daar de planten reeds vrij oud waren, hadden verschillende ervan reeds vergelend tot afstervend blad. Van de geoogste knollen werd de kiemrust één week na de tweede rooidatum door middel van een rindite-behandeling gebroken. Hierna werden de knollen bij een constante temperatuur van 20 °C bewaard. Drie, vier en acht weken na het breken van de kiemrust werden de knollen op aanwezigheid van Y^N-virus onderzocht door middel van spruittoetsingen, voor zover een spruit van minstens $1^1/_2$ cm lengte aanwezig was. Verder werd nagegaan in hoeverre sap van knollen als inoculum ter onderkenning van het Y^N-virus kon worden gebruikt. Vijf weken na het breken van de kiemrust werden navel- en krooneinden van de knollen op A6-blad getoetst. De toetsingen van de naveleinden mislukten toen. Acht en tien weken na het breken van de kiemrust werden de naveleinden der knollen nogmaals getoetst op aanwezigheid van Y^N-virus.

Vijf weken na het breken van de kiemrust werd van elke knol één spruit in de kas uitgeplant. De uit deze spruiten opgegroeide plantjes werden tien weken na het breken van de kiemrust op de gebruikelijke wijze getoetst. Tenslotte werden alle knollen in een aantal stukken gesneden, gelijk aan het aantal ogen, en in de kas uitgeplant. De uit deze ogen opgegroeide plantjes werden eveneens op aanwezigheid van Y^N-virus getoetst. Door deze toetsing was het mogelijk een verdeling te maken tussen partieel en geheel besmette knollen. Het viel hierbij op dat de planten, die op het moment van inoculatie vergelend tot afstervend blad hadden, knollen produceerden, die partieel besmet waren. Nadat een scheiding was gemaakt tussen geheel en partieel besmette knollen, werden de resultaten nagegaan van de toetsingen, waarbij gekneusde spruiten en aangesneden knollen als inoculum werden gebruikt.

In tabel 5 zijn de resultaten weergegeven van het onderzoek op aanwezigheid van Y^N-virus in de knol, waarbij gekneusde spruiten als inoculum werden gebruikt. Per knol is steeds één spruit getoetst. Van de geheel besmette knollen kon bij de negen dagen na de inoculatie gerooide knollen reeds voor 80 tot 91 % de besmetting worden aangetoond. Van de na 14 dagen gerooide knollen kon de besmetting voor 100% worden achterhaald. Zoals uit de gegevens blijkt, kon de aanwezigheid van het Y^N-virus in partieel besmette knollen slechts voor een gering percentage worden vastgesteld.

TABEL 5. Percentages knollen (ras Bintje), waarin Y^N-virus werd vastgesteld door middel van A6-bladtoets, indien spruitsap als inoculum was gebruikt.
Percentage of infected tubers detected by means of A6-leaf test, using sprout sap as inoculum.

Aantal dagen tussen inoculatie en rooien *Interval in days between inoculation and harvesting*	Aantal weken na het breken van de kiemrust *Number of weeks after breaking dormancy*	Aantal spruiten waarin Y^N-virus werd vastgesteld bij geheel besmette knollen[1] *Number of infected sprouts detected on completely infected tubers*[1]	Aantal spruiten, waarin Y^N-virus werd vastgesteld bij partieel besmette knollen[1] *Number of infected sprouts detected on incompletely infected tubers*[1]
9	3	12/15 = 80%	0/2 = 0%
	4	9/10 = 90%	0/3 = 0%
	8	22/24 = 91%	1/6 = 16%
14	3	11/11 = 100%	1/11 = 9%
	4	15/15 = 100%	3/10 = 30%
	8	17/17 = 100%	5/16 = 31%

[1] Teller = aantal spruiten, waarin Y^N-virus werd aangetoond.
Noemer = totaal aantal spruiten.
[1] *Numerator = number of infected sprouts detected by test.*
Denominator = total number of sprouts tested.

In tabel 6 zijn de resultaten weergegeven van proeven, waarbij aangesneden knollen als inoculum werden gebruikt. Van de geheel besmette knollen kon steeds het hoogste percentage worden aangetoond, als het basale aangesneden deel als inoculum werd gebruikt. Voor de partieel besmette knollen is het verloop in de verkregen gegevens grillig. Vijf en acht weken na het breken van de kiemrust werd met toetsen van het naveleinde het hoogste percentage zieke knollen gevonden, terwijl daarentegen 12 weken na het breken van de kiemrust het toetsen van de krooneinden het hoogste percentage opleverde. Daar

echter in dit geval met kleine aantallen is gewerkt, mag niet te veel waarde worden gehecht aan de resultaten van de partieel besmette knollen.

TABEL 6. Aantal knollen (ras Bintje) waarin het Y^N-virus werd vastgesteld door middel van de A6-bladtoets, waarbij aangesneden knollen als inoculum waren gebruikt.
Number of infected tubers detected by means of A6-leaf test using cut tubers as inoculum.

Aantal dagen tussen inoculatie en rooien *Interval in days between inoculation and harvesting*	Aantal weken na het breken van de kiemrust *Number of weeks after breaking dormancy*				Mate van knolbesmetting *Extent of tuber infection*
	5	8	12	12	
	Krooneinde[1] *Eye end*[1]	Naveleinde[1] *Heel end*[1]	Krooneinde[1] *Eye end*[1]	Naveleinde[1] *Heel end*[1]	
9	30/43 = 70%	38/43 = 88%	15/22 = 68%	25/31 = 80%	geheel *completely*
	2/10 = 20%	6/10 = 60%	1/5 = 20%	0/5 = 0%	partieel *incompletely*
14	11/29 = 40%	26/29 = 90%	13/23 = 56%	17/23 = 74%	geheel *completely*
	2/22 = 9%	7/22 = 30%	4/20 = 20%	2/20 = 10%	partieel *incompletely*

[1] Teller = aantal knollen, waarin Y^N-virus werd aangetoond.
Noemer = totaal aantal knollen.
[1] *Numerator = number of infected tubers detected by test.*
Denominator = total number of tubers tested.

NABESCHOUWING

Uit de proeven blijkt, dat in het algemeen aangesneden aardappelknollen, die nog in de kiemrust verkeren, niet als inoculum voor de bladtoets kunnen worden gebruikt.

Naarmate echter het tijdstip van het rooien later na de inoculatiedatum wordt gekozen, kan op het moment van het rooien een hoger percentage van de besmette knollen door middel van de bladtoets worden vastgesteld. Voor de kasomstandigheden was de tijdsduur tussen inoculeren en rooien, waarbij in ongeveer $^3/_4$ van het aantal besmette knollen het virus kon worden vastgesteld, 42 dagen, niettegenstaande het virus na zeven dagen de knol reeds had bereikt (tabel 4). Het blijkt, dat spruitende, aangesneden knollen – de kiemrust der knollen was kunstmatig gebroken – met goed resultaat als inoculum kunnen worden gebruikt. In hoeverre het breken van de kiemrust hierop een gunstig effect heeft, is niet bekend. MÜNSTER (niet gepubliceerd) heeft evenwel aanwijzingen, dat het breken van de kiemrust door middel van behandeling met rindite de virusconcentratie in de knol aanzienlijk doet stijgen. Volgens de gegevens uit tabel 3 wordt bij vier weken bewaren zonder het breken van de kiemrust ook reeds een hoger percentage besmette knollen door middel van de bladtoets gevonden dan vlak na het rooien. Er dient echter te worden opgemerkt, dat in het laatste geval de proefplanten jonger waren, zodat vergelijking met de resultaten uit tabel 4 niet geheel geoorloofd is.

Het is nog een vraag, wat er tijdens de bewaring en kieming van de knollen gebeurt. Een virusvermeerdering lijkt het meest waarschijnlijk. Het is echter ook denkbaar dat er geen virusvermeerdering plaats heeft, maar een vermin-

dering van de remstof, die door NIENHAUS (1960) kon worden aangetoond. Het effect is in beide gevallen gelijk.

Ook ten aanzien van hetgeen zich gedurende de eerste tijd van de besmetting in de knol afspeelt zijn er nog duistere punten. Aangezien het apicale deel van de knol het jongste en daardoor het actiefste deel is, is het denkbaar, dat het virus in de eerste plaats daarheen wordt getransporteerd en zich daar vermeerdert. Dit zou dan – ook volgens NIENHAUS (mondelinge mededeling) – hoofdzakelijk plaatshebben bij jonge, snel groeiende knollen. Bij oudere knollen, die op een laat tijdstip worden besmet, is de activiteit veel geringer en zal daardoor het virus hoofdzakelijk in het naveleinde van de knol achterblijven en het krooneinde niet meer kunnen bereiken. Deze hypothese kan echter niet worden gestaafd door de verkregen resultaten van de partieel besmette knollen.

Om de virusconcentratie in de verschillende knolgedeelten na te gaan, werd bij vele toetsingen het aantal necrotische kringen geteld, die bij inoculatie op het A6-toetsblad ontstonden. Hoewel deze tellingen geen absolute maat voor de virusconcentratie zijn, kunnen zij wel enige aanwijzing geven. Het aantal necrotische kringen per blad bij gelijk inoculum is nl. bij A6 zeer variabel; een betere toetsplant voor deze tellingen is volgens NIENHAUS (mondelinge mededeling) *Physalis floridana*. Bij de spruittoetsingen was het aantal necrotische kringen op A6-blad, vergeleken met toetsingen van de knol waarvan de kiemrust was gebroken, ongeveer 10 maal zo groot. Bij de toetsingen van de twee uiteinden der knol konden op A6-blad geen grote verschillen in aantallen necrotische kringen worden aangetoond. MÜNSTER (niet gepubliceerd) en NIENHAUS (niet gepubliceerd) vonden bij knollen waarvan de kiemrust was gebroken aan het apicale deel van de knol een hogere virusconcentratie dan aan het basale deel. Beiden hebben echter knollen onderzocht, waarvan nadere gegevens, zoals leeftijd bij infectie en rooidatum na infectie, ontbreken.

In tegenstelling met de bevindingen van NIENHAUS (1961) gaven spruittoetsingen steeds goede overeenstemming met het werkelijk aanwezig zijn van het virus. In hoeverre de verschillen in de rassen van het Duitse en Nederlandse aardappelsortiment hierbij een rol spelen, is nog niet onderzocht.

SAMENVATTING EN CONCLUSIES

Om de mogelijkheid van het gebruik van aangesneden knollen als inoculum voor de bladtoets na te gaan, werd een aantal proeven uitgevoerd met aardappelplanten van de rassen Bintje en Record. Door inoculaties uit te voeren op verschillende tijdstippen na het planten en de geïnoculeerde planten op verschillende data na inoculatie te rooien, werd enig inzicht verkregen in de mogelijkheid van toetsingen van knollen, die op verschillende tijden na inoculatie werden gerooid.

Het blijkt, dat aangesneden, in rust verkerende knollen met de hier beschreven methode niet als inoculum kunnen worden gebruikt, tenzij de planten 30 dagen of later na inoculatie worden gerooid. Vindt het rooien op een vroeger tijdstip na de inoculatie plaats, dan kunnen incidenteel enkele van de besmette knollen met de bladtoets worden gevonden. Indien de toetsing van besmette knollen tenminste één maand na het rooien wordt uitgevoerd, dan kan, indien de planten op het moment van de inoculatie niet te oud zijn geweest, het virus in ongeveer 50% van de besmette knollen worden aangetoond. Wordt echter

de kiemrust der knollen door middel van rindite kunstmatig gebroken en worden de knollen hierna gedurende enige weken bij 20 °C bewaard, dan wordt hetzelfde effect verkregen als bij bewaring onder natuurlijke omstandigheden. Met de rindite-behandeling wordt dit effect echter sneller bereikt. Uit de genoemde proeven blijkt, dat vijf weken na het breken van de kiemrust het virus in ongeveer 90% van de besmette knollen werd gevonden, indien de aangesneden naveleinden van geheel besmette knollen getoetst waren. Partieel besmette knollen geven geen betrouwbare resultaten.

Worden van geheel besmette knollen, waarvan de kiemrust kunstmatig is gebroken, minstens drie weken na het breken van de kiemrust de gekneusde spruiten als inoculum op A6-blad getoetst, dan kunnen 90 tot 100% der besmette knollen worden aangetoond.

Door tellingen van de necrotische kringen op het A6-blad werd voorts geconstateerd, dat de virusconcentratie in spruiten, zowel voor de knollen waarvan de kiemrust op kunstmatige als op natuurlijke wijze is gebroken, steeds hoger is dan de concentratie in het apicale en het basale deel van de knol.

Summary

The possibility of detecting virus Y^N infections in potato tubers by means of A 6 and SdY leaf tests was examined. Infected tubers were obtained by inoculating plants of the varieties Bintje and Record at various intervals after planting and by harvesting at different intervals after inoculation.

It was found that cut dormant tubers cannot be used for testing on detached leaves unless the period between inoculation and harvest is longer than 30 days (tables 1, 2, 3). If testing of the tubers is withheld until they have been in storage for at least one month, it then becomes possible to detect about 50% of the infections (table 4). Successful detection of the infected tubers thus depends largely on the age of the plants at the moment of inoculation and the time interval between inoculation and harvesting. By breaking the dormancy of the tubers artificially with "rindite" and then storing them for some weeks at 20 °C, the same effect can be achieved as when the tubers are allowed to sprout naturally. By means of the "rindite" treatment, however, the desired condition can be obtained much earlier. It is not yet clear whether this increase in detectable virus is due to virus multiplication or to diminishing inhibitor concentration.

Experiments showed that if the heel ends of cut, completely infected tubers are tested five weeks after breaking dormancy, about 90% of the infected tubers can be detected. Partially infected tubers do not give reliable results (table 6).

A higher percentage of the infected tubers can be detected when sap expressed from sprouts is used as inoculum for the A6-leaf test three weeks or more after breaking dormancy (table 5).

It was noticed that the virus concentration in the sprouts of tubers in which dormancy had been broken artificially or naturally was always higher than that in the apical or basal parts of the same tubers.

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Potato viruses and some remarks on their control

by

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POTATO VIRUSES AND SOME REMARKS ON THEIR CONTROL

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1. INTRODUCTION

Since Quanjer in 1916 demonstrated that potato degeneration was primarily attributable to an infectious disease and Oortwijn Botjes in 1920 proved that leaf roll could be transmitted by aphids, work on virus diseases has been in progress on a large scale all over the world. Many aspects have been investigated, and now, after about fifty years of intensive work, at least twenty different viruses are known to affect the potato. They are not all of equal economic importance but they show the complexity of the potato virus problem.

Although it can be claimed that increased knowledge of the modes of transmission and many other aspects of viruses has often led to successful control and thus considerably diminished the damage brought about by them, we are still often faced with great difficulties. These may be caused by the appearance of new viruses or virus strains, by the fact that a disease hitherto not identified is found to be attributable to a virus, or by abnormal environmental conditions. Research on potato viruses, therefore, is still increasing and often leads to new possibilities for more efficient control.

It is proposed here to discuss problems of current interest in potato virus research. To begin with, some characteristics of the most important potato viruses will be given, with special reference to those facts which have been established recently. Within the time available it is not possible to give a full description of symptoms so that in most instances only a brief indication will be given of the type of symptoms produced. This will be followed by a survey of methods of diagnosis and a discussion of methods used in the control.

I shall restrict myself for the greater part to those problems which are of practical importance. It will of course be impossible to give a systematic review without mentioning certain facts which have been known for a long time.

2. VIRUSES AFFECTING THE POTATO

2.1. Viruses transmitted by aphids

2.1.1. Leaf roll virus

One of the most important and well-studied potato viruses is that of leaf roll. It has a world-wide distribution occurring wherever potatoes are grown. Characteristic is the upward rolling of the edges of the lower leaves and the erect appearance of the whole plant, accompanied by necrosis of the phloem and accumulation of starch in

the leaves. The depression in yield may be considerable and depends to a large extent on variety and on climatological conditions. Total infection will lead on the average to a yield decrease of about 50% although in extreme cases it may almost destroy the crop completely.

Under natural conditions the virus is transmitted by aphids of which *Myzus persicae* is considered to be the most important. The virus is persistent in its vector which means that the total process of transmission takes considerable time, viz. about two days. Incidently, transmission within relatively short period could be detected (KLOSTERMEYER, 1953; DE MEESTER-MANGER CATS, 1956) using hosts other than potato. It is of interest to note that the leaf roll virus multiplies in its vector *Myzus persicae*. This has been demonstrated by injecting aphids with suspensions from leaf roll infected plants or from viruliferous aphids (HEINZE, 1955; STEGWEE and PONSEN, 1958). Such a technique may be of use in solving a number of problems connected with leaf roll and other persistent viruses. Hitherto, multiplication of plant viruses in their vectors had been reported only for leafhopper-borne viruses.

2.1.2. Potato virus Y

The second virus to be mentioned is potato virus Y, which is also wide-spread. Infected plants may be symptomless or show highly necrotic reactions depending on the strain of the virus, the potato variety and environmental conditions. The virus is aphid-transmitted and non-persistent in its vector, which means that aphids need only very short acquisition and infection feeding periods for the transmission of the virus. Yield depression is considerable; for old strains it can average as much as 50 %.

A new strain – the tobacco veinal necrosis strain – has spread rapidly in Western Germany during the last few years and also, since 1959, in Holland. ARENZ and HUNNIUS (1959) estimated the losses brought about by this new strain and found a yield depression of about 14% in masked infections and of about 20% in visible infections. The control of this strain presents great difficulties and, therefore, it is one of the major virus problems in the seed potato growing areas of Western Germany and Holland. Characteristic is that it shows relatively mild symptoms, which can easily be overlooked and this undoubtedly is one of the factors responsible for the rapid spread of the virus in recent years.

Virus Y can be transmitted by a number of aphid species of which *Myzus persicae* is the most effective under glasshouse conditions. VÖLK (1959) investigated some characteristics of the transmission of the tobacco veinal necrosis strain and concluded that under natural conditons aphid species other than *Myzus persicae* may be of importance as well. He mentions *Doralis rhamni* and *Doralis frangulae* as aphid species frequently occuring in potatoes and which were found to be good vectors of the virus. Moreover it was discovered that the tobacco veinal necrosis strain of virus Y can be transmitted mechanically by contact and this possibly plays a role in the epidemiology of the virus.

2.1.3. Potato virus A

Hitherto potato virus A, which as a rule causes only a mild mosaic, has not been studied as thoroughly as the foregoing viruses. It is aphid-transmitted and non-persistent in its vector and the damage it causes is relatively small. Virus A is generally considered to be distinct from all other potato viruses, although recently COCKERHAM (1958) has obtained evidence that it may bear some relationship to the group of Y-viruses.

2.2. Viruses transmitted by leafhoppers

A virus disease which is of great economic importance in potatoes in Eastern Europe and which is being studied especially in Czecho-Slovakia is that caused by the (tomato) stolbur virus. According to KLINKOWSKI (1958) this disease is spreading in a western direction.

As many features of the disease are still problematic we shall mention only some of its most striking characteristics. The leafhopper *Hyalesthes obsoletus* transmits the virus under natural conditions and there are indications that it can be transmitted also by other species (VALENTA, 1958). The virus spreads rapidly in the field, but it depends on the strain of the virus involved and on environmental conditions whether the tubers of infected plants become infected or not. The virus has a wide host range and may be related to some viruses causing witches' broom-like diseases in the U.S.A. and some other countries. In potato, the first symptoms consist of a chlorotic discoloration of the leaf edges and a spoon-like rolling of the leaves after a short period followed by wilting and desiccation. The water content of the tubers decreases gradually and the tubers become soft. Such tubers form spindle sprouts which, however, are not exclusively a characteristic of stolbur.

2.3. Soil-borne viruses

A special group of potato viruses comprises those which are soil-borne. Until recently the transmission of virus through the soil was generally regarded as a relatively unimportant mode of spread, but the results of recent work done in various parts of the world have shown that this is not the case.

QUANJER (1926), working with spraing or corky ringspot (Dutch: kringerigheid) was the first to suggest that a soil-borne virus might be the cause of the disease. It is a disorder of the tubers, which develop internally brown, necrotic rings in various patterns. It occurs only on sandy and peaty soils and is often limited to special areas in a field. ROZENDAAL and VAN DER WANT (1948) formed the hypothesis that spraing is caused by a strain of potato stem-mottle virus, which was already known to be soil-borne (ROZENDAAL, 1947). Recently various workers (LIHNELL, 1957; WALKINSHAW and LARSON, 1958; OSWALD and BOWMAN, 1958; CADMAN, 1959; BRANDENBURG, EIBNER and TOSTMANN, 1959) found strong evidence that spraing is indeed caused by a virus related to the potato stem-mottle virus. LIHNELL (1958) described spraing

mosaic as a symptom sometimes appearing in potato plants grown from spraing affected tubers. This spraing mosaic resembles in many respects that caused by potato stem-mottle, viz. only a few stems of a plant are stunted and weak in appearance with leaves showing a more or less pronounced mottle. Another point of interest described by LIHNELL is that primary and secondary spraing can be distinghuished.

Primary spraing consists of rings near the skin of tubers through which the virus apparently entered, whereas secondary spraing consists of rings in the tuber near the heel end which suggest that the virus entered through the stolon from the mother plant.

Stem-mottle virus also occurs on sandy and peaty soils and infects many crops and wild plants. Often it is only detectable in the roots of infected weeds (NOORDAM, 1956; CADMAN and HARRISON, 1959). It is characteristic that the next tuber generation of an infected plant is often only partially infected or even virus free, which is also the case with spraing. The causal virus, a strain of which causes the tobacco rattle disease (ROZENDAAL and VAN DER WANT, 1948) is strongly adsorbed on soil colloids and, according to VAN DER WANT (1953), this may be the reason why the virus can remain infective in the soil for very long periods. It is not clear, however, why soil-borne viruses do not occur on heavy clay soils where there is an excess of soil colloids. On the other hand VAN DER WANT stated that a soil-inhabiting organism may possibly play a role in the survival of the virus. Recent work on the tobacco rattle disease has shown that indeed an eelworm *(Trichodorus pachydermus)* can transmit tobacco rattle virus (SOL, VAN HEUVEN and SEINHORST, 1960). The finding of an animal vector of this virus, which perhaps will lead to similar findings with regard to other soil-borne potato viruses, offers possibilities for solving many of the problems connected with this group of viruses.

Other soil-borne viruses which may be of importance in potatoes are those of the tobacco ringspot group. Recently HARRISON (1958) described a potato disease caused by the beet ringspot virus which was found to be soil-borne and which is possibly related to the bouquet virus occurring in Germany (KÖHLER, 1952). However, to my knowledge, soil transmission of the bouquet virus has not been demonstrated in Germany. As is the case with potato stem-mottle and related viruses, the beet ringspot virus is restricted to sandy and peaty soils and is not transmitted 100% to the next tuber generation. It is at present only known to occur in Scotland and Germany but may have escaped detection elsewhere, for the ringspot group as a whole is known to be widespread.

2.4. Viruses transmitted mechanically

Yet another group of viruses comprises those which are transmitted by contact. The most important member of this group is potato virus X, which gives rise to a more or less distinct mosaic in most potato varieties. There are, however, varieties which act as symptomless carriers, e.g. *Duke of York*, which is infected 100% with a strain of this virus. Its transmission in the field occurs mechanically, e.g. by the rubbing of the

leaves by the wind. It is also readily transmissable on the clothes of persons and on the fur of animals (TODD, 1958).

Transmission by root contact seems to be of minor importance (BARTELS, 1953).

To this group belongs also virus S which generally causes rather mild symptoms, consisting of mild mosaic in some varieties and slight alterations in the habit of growth (ROZENDAAL and BRUST, 1955). The viruses X and S are present in many parts of the world and older varieties especially may be infected to a high degree in regions where no control measures have been taken.

A virus which recently has attracted some attention is potato virus M which, as far as mode of transmission is concerned, belongs to the group of aphid-transmitted viruses. Recent work on the serology and electronmicroscopy of the viruses S and M has shown, however, that these two viruses have many characteristics in common. It has been suggested that virus S originated from virus M but that it lost the characteristic of transmissibility by aphids (BRANDES, WETTER, BAGNALL and LARSON, 1959).

3. METHODS APPLIED IN THE DIAGNOSIS OF POTATO VIRUSES

Effective control of potato virus diseases is only possible if we are well informed as to the causal viruses and their modes of transmission. For the application of control measures, simple techniques for diagnosing which virus is present are an indispensable pre-requisite. We will therefore discuss some of the methods which are either in use at present or have been developed recently.

Testing of the growing plants for the presence of virus must continue from the time of emergence until harvest. During the first part of this period infected plants must be removed in order to eliminate any possible source of infection for the healthy plants. In many cases, experienced persons by watching for symptoms can point out those plants which have been grown from infected tubers. Such plants can then be removed. However, in cases of latent infection or where symptoms appear late due to unfavourable circumstances, special methods must be applied to trace the infected plants.

Generally speaking, leaf roll and old virus Y strains do not offer difficulties in diagnosis when the plants have grown from infected tubers. However, the viruses X, S and the tobacco veinal necrosis strain of virus Y are often difficult to distinguish and serological or test-plant methods have to be employed. As is described by VAN SLOGTEREN (1959) by means of polyvalent antisera in an agglutinin test the viruses X, S and M can nowadays be serologically diagnosed in one single test. This method, which gives immediate results allows us to investigate large numbers of plants very rapidly.

As the tobacco veinal necrosis strain of virus Y often gives rise to masked infections and serological testing has not always proved completely satisfactory, we have in this case to resort to the use of test plants. Any plant that gives distinct symptoms after inoculation with a given virus can serve as a test plant. For practical purposes it is preferable to have a plant which reacts with local, necrotic lesions on the inoculated leaves as, generally speaking, necrotic reactions appear much sooner than systemic

ones. Moreover, the method demands little space, as detached leaves can be used. Such test plants for the new strain of virus Y (and also for old strains) are the potato seedling A6 introduced by KÖHLER (1953) and a special line of *Solanum demissum* designated as SdY, introduced by COCKERHAM (1958). Recently DE BOKX (1960) compared the results obtained with these two test plants and found that for routine work SdY is the most suitable, best results being obtained with leaves in the dark, at a high humidity and at a temperature of about 20°C. A point of interest, especially for routine work, is the fact that A6 does not give satisfactory results in the dark.

Virus diagnosis in the case of growing plants at a later stage of growth concerns primarily those plants which become infected during the growing season. An exact diagnosis at this stage could enable us to predict the degree of infection of the tubers at the time of harvesting. Early infections can often be detected by watching for symptoms, but none of the methods of diagnosis hitherto applied have proved sensitive enough to detect every plant that became infected during the latter part of the growing period. It is, therefore, very difficult to predict with certainty the state of health of the tubers from the degree of infection of the foliage just before harvest.

It follows, therefore, that in order to determine whether the tubers are infected or not, the tubers themselves must be examined. By taking samples – the numbers of tubers to be taken is a problem in itself which we will pass over for the moment – we can try, in one way or another to estimate the number of infected tubers. For practical reasons this should preferably be done as soon as possible after harvesting. It would, therefore, be most desirable to be able to test the tubers by a method which gives immediate results. So far no easy way has been found to diagnose serologically the presence of viruses within potato tubers. This is probably because the concentration of virus is too low or because certain substances inhibit the serological reaction. In this connection it is of interest to remark that for the viruses X and Y, and to a lesser degree for leaf roll also, it has been found that tubers of primarily infected plants may become partially infected. This means that healthy and infected parts are present in one tuber, which suggests that a relatively small concentration of virus is present in the tubers (BEEMSTER, 1958 a, b, 1960). Moreover, NIENHAUS (1960) describes the existence of a substance in the outer layer of potato tubers which strongly inactivates virus. NIENHAUS developed the method of rubbing special parts of the tubers on to tobacco, which plant becomes systemically infected. This gives fairly good results. It would, however, be of interest to know whether local lesion hosts can be used as well. The disadvantage of using hosts which react systemically has already been mentioned earlier. It must be noted that NIENHAUS could not obtain satisfactory results with dormant tubers but only after the tubers had been sprouting for about two weeks. This is most probably due to the activation of virus multiplication following an inability to multiply during the dormant stage.

For some years now it has been possible to detect leaf roll virus quickly by means of the callose test. This test is based upon the fact that in leaf roll diseased plants and tubers an accumulation of callose takes place in the phloem cells and that this callose can be made visible by staining with resorcine blue. Although this method does not

make it possible to detect each infected tuber, a reasonably good estimation of the percentage of infected tubers can be obtained. As this test can be applied only in the case of leaf roll it is worth mentioning that RUSCHKE-HEILMANN (1960) recently published a method purporting to enable the detection of the viruses X, S, Y, leaf roll and bouquet in potato tubers on the basis of size differences which arise in phloem cells infected with the respective viruses. At the moment no data are available as to whether this test can be adapted for practical purposes.

In estimating the value of a test on potato tubers we must always be aware of the fact that it is unlikely that any test will be reliable because, as was stated earlier, in the case of late infections the concentration of a virus within the tubers shortly after harvesting may be very low. It would be worthwhile looking for methods of enhancing the virus titer within the tubers in order to improve existing test methods or the possibilities of finding new methods.

An obvious means of obtaining higher concentrations of virus is to plant the tuber. Diagnosis is then possible by testing the leaves of the sprouts by one of the diagnostic methods described earlier. This so-called tuber- or eye-index method is widely used but has the disadvantage that the production of sprouts takes rather a long time. Moreover, glasshouses are required which in many cases considerably limits the capacity of the test. Fortunately we can utilize the Rindite method to break dormancy of the tubers for otherwise it would be necessary to wait at least another 6–8 weeks after harvesting before the tubers would start growing.

Only in the case of virus X a serological diagnosis is possible on sprouts grown in the dark. In applying this method no glasshouses are required. It would be worth trying to develop a similar method using sprouting tubers for the serological diagnosis of virus Y also.

Theoretically, there is the possibility of approaching the diagnosis problem biochemically. However, the question is whether or not the synthesis of viruses in infected plants gives rise to changes which can be detected biochemically. From past investigations it is known that differences between healthy and diseased plants exist, e.g. in the content of organic acids (VENEKAMP, 1959) and in the activity of enzymes (BOSER, 1958), but the differences found hitherto are of a quantitative rather than a qualitative nature; this makes a reliable diagnosis rather questionable.

4. THE CONTROL OF POTATO VIRUSES

The control of virus diseases in general can be achieved theoretically in two entirely different ways, viz. direct control which aims at the elimination of the virus from plants already infected, and indirect control which is based on prevention of infection. So far the latter method has proved by far the most important.

4.1. Direct control

A great obstacle in the way of direct control of plant viruses is that they generally

invade almost all plant tissues and, in order to cure infected plants, treatments must be applied which act systemically. Nowadays a number of substances of this kind are known, some of which can be applied successfully against insects, fungi or other micro-organisms. Unfortunately it has been proved that all substances which act against viruses within a plant are phytotoxic as well. Apparently the plant and virus constituents have so many sites in common that it is very difficult to find specific viricides. Therefore, it is obvious that all attempts to eliminate viruses from growing plants by the application of chemicals known to inhibit virus multiplication have been without success.

Some positive results against potato virus Y have, however, been obtained by spraying with thiouracil or trichothecin either shortly before or after inoculation (Bradley and Ganong, 1958; Bradley and McKinnon, 1957).

Heat treatment has been shown to inactivate a range of viruses and has been successfully used to free potato tubers from witches' broom and leaf roll viruses (Kunkel, 1943; Kassanis, 1950). This treatment has been without success against the viruses X and Y, S and stem-mottle (Rozendaal, 1952; Rozendaal and Brust, 1955). It is of interest that in India seed potatoes were cured of leaf roll by high temperature under natural conditions, temperatures of over 40°C being recorded. The tubers stored in cooled houses were not cured (Thirumalacher, 1954).

It is known that plant viruses invade certain plant tissues incompletely. This offers also a means of eliminating viruses from infected plants. It has been found that some viruses are not present in the apical meristematic region of their hosts and it is thus possible to obtain healthy plants by excising and regrowing this part of the plant. The method has been successfully used to free potato plants from viruses X, Y, A and paracrinkle (Morel and Martin, 1955; Kassanis, 1957). The procedure is very laborious but is of particular interest in freeing a potato variety stocks of which are all infected with a virus, e.g. the variety *Duke of York* which is 100% infected with both the viruses X and S. Quak (1958) succeeded in freeing this variety from X, but not so far from S. It has been proved that certain pre- treatments of the plants, e.g. spraying with thiouracil, increase the chance of obtaining healthy plants (Quak, 1958).

A more simple method has been described by Thompson (1958) who eliminated the viruses Y, A and S from potato plants by combining heat treatment with excision and regrowth of shoot apices. Virus Y could even be eliminated in some cases from cultures of stem apices grown at room temperature.

4.2. Indirect control

As direct control of potato viruses is as yet only possible to a limited extent, it is necessary to apply a number of other measures designed to keep potatoes virus free. In this respect it is most important to ensure that the tubers used for seed are free from virus. To carry out control measures effectively we must know the mode of transmission of the respective viruses under natural field conditions.

The first group to be discussed comprises those viruses which are transmitted only

by contact, viz. the viruses X and S. Control of these viruses is relatively simple since they can be diagnosed serologically. We can, therefore, look in a potato plot for one or more plants which are virus free and when such plants are found, they can be propagated in isolated areas to avoid infection. By repeated serological testing it is thus possible to obtain varieties which are completely free from the viruses X and S.

Control of those viruses which are aphid-transmitted involves far greater difficulties. In discussing this group of viruses we will restrict ourselves to leaf roll and virus Y, representing the groups of persistent and non-persistent viruses respectively.

In growing seed potatoes, measures must be taken to ensure that healthy plants do not become infected in the course of the growing season and that in those cases where infection does occur, the virus is prevented from entering the tubers. The simplest method is to grow the plant under such conditions that no sources of infection are present. This means that high quality seed potatoes should be produced only in large areas in which no ware potatoes are grown, since the latter crop often harbours large numbers of infected plants. This method can produce very good results (BOTHE, 1959), but there are difficulties which require special attention. It has so far rarely been possible to start with material which is completely free from viruses. Careful roguing must therefore be performed before any aphids are present to transmit viruses to the healthy plants. Another point of interest is that leaf roll virus can be brought into a field by aphids swept in by wind from distant areas. This results from the fact that aphids carrying leaf roll virus are able to transmit it throughout their lives. On the other hand, aphids loose their ability to transmit non-persistent viruses, such as virus Y, rather quickly. It can be concluded that although the growing of seed potatoes in isolated areas has certain advantages, both early roguing and early lifting must be applied as well.

As the growing of seed potatoes in isolated areas often meets with practical difficulties, we have to accept the fact that seed potatoes will continue to be produced in areas where infection from near-by fields remains a potential danger. In this situation success depends largely on the time of appearance and the activity of the aphid vectors which in turn depends on climatological conditions such as temperature, wind, possibilities for overwintering of aphids etc. As there are great differences between one year and the next and between regions, we will not go into details. We shall restrict ourselves to the common situation in Western Europe where aphids appear in large numbers at a certain moment after the tubers have been planted. Effective seed potato growing is of course not possible in those regions where aphids are present the whole year round.

It will be clear that for seed tuber production it is most important to start with healthy seed material or at least with only very few infected tubers. If some tubers are infected, the plants growing from them must be eliminated as early as possible. For the successful detection of all infected plants we need one or more of the diagnostic methods described earlier.

In spite of the minute care which may have been taken to counteract all sources of infection, it is not possible to evade completely each new infection. There may be

infected plants which have escaped detection or which are present in neighbouring fields. Moreover, other hosts than potato may be present from which viruses can be transmitted to potato. In the case of primary infections we have to try to prevent the virus from being translocated downwards to the tubers. The only means of achieving this is by harvesting the tubers at the right time. In this connection it is of interest to remark that, generally speaking, any plant that shows virus symptoms, yields only infected tubers. Unfortunately, however, this does not mean that a plant without visible symptoms yields only healthy tubers. From experiments we know that plants without any symptoms may yield a totally or partially infected crop of tubers.

The right time of early harvesting can be settled when we are well informed as to 1. the moment of appearance and the activity of the virus transmitting aphids and 2. the time the virus needs to move from the leaves to the tubers.

The presence and activity of aphids can be measured in different ways. We can take samples of a given number of leaves, on which the number of aphids is determined. In this way a good estimate can be obtained of the number of aphids present in a field. A method which is widely used to determine aphid activity, especially of the winged ones, is that of yellow traps (MOERICKE, 1951). From experiments it is known that winged aphids play a very important role in the transmission of viruses both from field to field and within a field. The role of apterous aphids is relatively small.

After having estimated the moment that aphids will become active in large quantities in the field, we need data regarding the speed of translocation of the viruses within the potato plant. From our work (BEEMSTER, 1958 a, b, 1960) we know that it is not possible to give an exact period between infection of leaves and that of the tubers because it is influenced by so many factors. In young plants, viruses can move from the leaves to the tubers within a week, but as the plants grow older the rate of movement is lower and can be so low that the virus may never reach the tubers at all. This so called mature plant resistance is certainly of importance in seed potato growing.

It is generally assumed that potatoes have to be harvested 10–14 days after infection of the leaves. In many cases this leads to a rather early date of harvesting, at which time the crop may not have reached its maximum yield, especially in the case of late potatoes. Sometimes it is not even possible to harvest at the time that it ought to be done. For this reason it is generally more difficult to grow high quality seed potatoes of late than of early varieties. From the foregoing it will be clear that it is very important to bring the plants to the resistant stage as early as possible by measures such as early planting and the use of pre-sprouted seed tubers. Furthermore it is desirable that all plants emerge simultaneously, as this facilitates early roguing.

A few other factors which may influence the development of the potato must be mentioned. It is well known that a too high supply of nitrogen to the soil does not favour the growing of seed potatoes. It keeps the above-ground parts growing, so that the plants stay in a young, susceptible stage for a long time. On the other hand phosphates, when applied at the right time, favour quick development and thus the plants reach the resistant stage at an early date. Without doubt, a number of other factors such as plant spacing, size of seed tubers and possibly pre-treatments of tubers,

influence to some extent the development of the plants. It would be very valuable to know of a characteristic feature which would reliably indicate that a potato plant had reached the stage of mature plant resistance. At present it is generally accepted that the time of flowering is an indication that the resistant stage has been reached but there may be better standards.

As was stated earlier, early harvesting is an essential procedure in seed potato growing. However, in practice it meets with many difficulties since it is not possible to harvest large areas within a few days. To start harvesting earlier is not very profitable as tuber growth is optimal just at this time. However, it is possible to remove the haulms very rapidly and thus prevent the virus from being translocated to the tubers. A number of methods of achieving this are known, e.g. pulling, pulverizing or killing the haulms chemically or mechanically. Pulling the haulms at the right time is considered to be the best. It is, however, very laborious as it can at present only be done by hand, although in the Netherlands, the development of a haulm-pulling machine has reached a promising stage. The second and most widely applied method is to kill the haulms chemically but this method has various disadvantages. In the first place, the chemicals which are generally used (arsenites and DNC) are very poisonous to men and animals. Many attempts have therefore been made to find a less poisonous haulm killer, but hitherto without much success. Perhaps the use of arsenites and DNC at low concentrations and in combination with chemicals which inhibit the development of new sprouts on the stems will be a preliminary solution. The second disadvantage of haulm killing is the fact that it has proved to be very difficult to obtain complete killing of the stems. It is known that if the stems are not thoroughly killed, second growth occurs which becomes easily infected and from which virus translocation downwards to the tubers is certainly possible. Therefore it can be stated that it is preferable not to attempt to kill the haulms at all rather than to kill them incompletely. A question of some importance is whether or not an increased downward movement of virus takes place during the period between spraying and the moment when the stems are completely killed. The experience of some potato growers in the Netherlands in 1959 lead to the conclusion that this is indeed probable, although it can not be explained on the basis of results from experiments on virus translocation. However, where possible, one should destroy the stems mechanically before applying chemicals.

As was pointed out above it is most important to eliminate all possible sources of infection in order to prevent virus spread when aphids appear. On the other hand it is quite obvious that the presence of infected plants is of little importance as a source of infection when no vectors are present or when they are not able to transmit the virus. Therefore, we have to pay some attention to the question of whether or not it is possible to prevent infection by controlling aphids. Regarding *Myzus persicae*, which is considered to be the most important aphid in the spread of viruses in potatoes, it would seem desirable to kill it on its winter hosts or better still, to eliminate these.

The most promising insecticides in aphid control are those which penetrate the plants and which act over a number of days. These so-called systemic insecticides

protect the plants from infestation by aphids for at least one week. In trying to control viruses by killing the aphids, however, we meet the difficulty that transmission of the virus may occur before the aphid is killed, especially in the case of non-persistent viruses such as virus Y where transmission takes only a few minutes. As was mentioned earlier, the transmission of leaf roll virus requires some considerable time and, therefore, the use of systemic insecticides offers good prospects for the control of this virus. Since the aphid is killed before the latent period has passed, the spread of leaf roll within a field can be completely prevented. On the other hand the spread of virus from near-by fields, which have not been sprayed, is possible because a viruliferous aphid can infect a sprayed plant before it is killed. From this it follows that spraying every potato field throughout large areas would be very desirable from the point of view of the control of leaf roll and would also be of some help in controling virus Y. A point of importance is that the first spraying must be carried out early even when only few aphids can be expected, as young, infected plants are a very good source of infection and young, healthy plants are much more susceptible than older ones.

When the tubers have been harvested it is not always possible to predict with certainty whether indeed a healthy crop has been obtained. To get a picture of the state of health of the tubers they should be tested by one of the diagnostic methods described above.

One may ask whether it is possible to obtain an idea of the extent of tuber infection by taking samples from the leaves or stems (for virus Y and leaf roll respectively) shortly before harvest. This procedure would be of great value because it would enable us to classify the tubers sooner than is possible by testing the tubers themselves. The reliability of such a test on leaf or stem samples is primarily a question of whether or not the virus concerned is detectable in the leaves at the moment that it is already present in the tubers. Our experience in virus translocation studies suggests that we have to be very careful here, as after infection at a rather late stage there may be only downward translocation of the virus to the tubers and not to any other leaf. It is nevertheless worth investigating this problem as perhaps certain correlations do exist between the rates of leaf- and tuber infection.

An attractive possibility is to reduce the damage caused by viruses in potatoes by the breeding of resistant varieties. Although we are sure that this is by no means the least important possibility, we will conclude with only a few remarks on this very specialized aspect of the potato virus problem. In the existing potato varieties there are already great differences in susceptibility and sensitivity to the various viruses. The most desirable characteristics are of course complete immunity or hypersensitivity (field immunity). In the latter case the virus, after being introduced into the plant, does not become systemic. Although much work has been done on the breeding of virus-resistant varieties it has proved to be very difficult since there are a great number of characteristics which determine whether or not a new variety can be substituted for one which is already generally accepted. However, the results obtained from breeding work done so far suggest that, within the near future, the growing of varieties which are either immune or hypersensitive to one or more viruses, will increase in importance.

In this paper I have tried to give a survey of the possibilities which exist for the control of potato virus diseases. In doing so I have paid special attention to those viruses which are transmitted by aphids since their occurrence is the most general. Time did not permit me to give details or even discuss the control of some types of viruses, e.g. those which are soil-borne. Nevertheless I hope I have made clear just what difficulties and possibilities there are.

The potato as a food crop is of world-wide significance. Its vegetative propagation and the fact that seed potatoes move from one country to another and even from continent to continent necessarily imply that virus problems arising in a certain region and which seem to be only of regional interest, are in fact – in many cases – of universal importance. The occasional occurrence of a hitherto unknown virus to-day may be a serious problem in the world of to-morrow. Research workers in any part of the world should be conscious of this fact, and close collaboration among them must be considered to be of the utmost importance.

Fortunately it can be stated that the exchange of new information among workers on potato viruses has been very common now for some time. Since constantly there is the danger of introducing new viruses intro countries where previously they were not present, the exchange of ideas and new information is generally considered to be of the utmost importance. The fact that potato virologists from many countries now meet for the fourth time within ten years seems to me to be unequivocal affirmation of this statement.

Even better collaboration would be possible if what MARAMOROSCH outlined in a resolution at the IVth International Congress of Crop Protection at Hamburg in 1957 could be realized. He proposed the establishment somewhere of an international institution where viruses from all over the world could be gathered and investigated. Such an institution would greatly facilitate the solving of existing problems, especially those regarding the identity of viruses, and would be also of great practical value in improving potato virus control all over the world.

SUMMARY

Although knowledge of viruses and virus diseases of potatoes has increased greatly during the last fifty years, many problems still exist. A pre-requisite for efficient control is to be well informed regarding the mode of transmission of each virus. In this respect potato viruses can be divided into different groups: viz. viruses transmitted mechanically by contact, by aphids, by leafhoppers and the soil-borne viruses. There are indications that nematodes play a role in the transmission of the soil-borne viruses.

It is most important to be able to diagnose the presence of virus easily. The application of existing methods and the prospects for their further development and improvement are outlined, together with some of the newer methods which have been developed recently and are not yet in general use.

The control of potato viruses by direct methods, viz. the elimination of virus

already present in a potato plant or tuber by means of heat treatment and/or meristem culture, is possible in some instances. Until now, however, these methods have proved of use only in cases where a potato variety is totally infected by a virus and they can be used to free the variety as a whole from the virus.

Indirect measures still form the most important aspect of potato virus control. A description is given of measures that can be taken in order to obtain healthy seed tubers. Some of these are: early roguing, early harvesting, the use of pre-sprouted tubers and the application of all other suitable measures designed for bringing the plants to the stage of mature plant resistance as early as possible. The use of systemic insecticides to kill aphid vectors and the breeding of immune or resistant varieties are considered as very important aids for the efficient control of potato viruses.

ZUSAMMENFASSUNG

KARTOFFEL-VIRUSKRANKHEITEN UND EINIGES ÜBER IHRE BEKÄMPFUNG

Obwohl sich unsere Kenntnisse von Viren und Viruskrankheiten von Kartoffeln in den letzten fünfzig Jahren bedeutend erweitert haben, harren noch viele Probleme der Lösung. Für eine wirksame Bekämpfung ist eines der Grunderfordernisse, die Art der Uebertragung der einzelnen Viren gut zu kennen. In dieser Beziehung lassen sich Kartoffelviren in mehrere Gruppen einteilen, und zwar Viren, die mechanisch durch Kontakt, Viren, die durch Blattläuse, oder durch Zikaden, übertragen werden, und die Bodenübertragbare Viren. Es sind Anzeichen dafür vorhanden, dass Nematoden an der Uebertragung der letztgenannten Viren beteiligt sind.

Sehr wichtig ist es, die Anwesenheit von Viren leicht erkennen zu können. Die Anwendung bestehender Verfahren und die Aussichten für ihre Weiterentwicklung und Verbesserung werden erörtert, zusammen mit einigen der neueren Methoden, die in letzter Zeit ausgearbeitet worden sind und noch nicht allgemein Anwendung finden.

Die Bekämpfung von Kartoffelviren durch direkte Methoden, d.h. die Ausmerzung von bereits in einer Kartoffelpflanze oder -knolle anwesendem Virus durch Wärmebehandlung und/oder Meristemkultur, ist in einigen Fällen möglich. Bisher jedoch hat die Anwendung dieser Methoden sich nur gebräuchlich gezeigt in Fällen, wo eine Kartoffelsorte vollkommen durch einen Virus infiziert war, und sie daher dazu dienen, die Sorte als Ganzes virusfrei zu machen.

Indirekte Methoden sind bei der Kartoffelvirusbekämpfung noch immer am wichtigsten. Es wird eine Beschreibung der Massnahmen gegeben, die getroffen werden können, um gesunde Knollen zu erzielen. Einige derselben sind: frühzeitiges Jäten, Frühernte, Verwendung vorgekeimter Knollen und Anwendung aller sonstigen geeigneten Massnahmen, die Pflanzen so bald wie möglich in das Stadium der Altersresistenz zu bringen. Die Anwendung von systemisch wirksamen Insektiziden zur Abtötung der virusübertragenden Blattläuse und die Züchtung immuner oder resistenter Sorten werden als sehr wichtige Hilfsmittel zur wirksamen Bekämpfung von Kartoffelviren bezeichnet.

RÉSUMÉ

MALADIES A VIRUS DE LA POMME DE TERRE ET QUELQUES REMARQUES SUR LES MOYENS DE LUTTE

Bien que les connaissances sur les virus et les maladies à virus de la pomme de terre se soient largement accrues ces cinquante dernières années, il reste encore bien des problèmes. Pour lutter d'une façon efficace contre ces maladies, il est indispensable de bien connaître le mode de transmission de chaque virus. Sous ce rapport, les virus de la pomme de terre se classent en différents groupes: les virus transmissibles par voie mécanique par contact, les virus transmissibles par les pucerons, les sauterelles et les virus conservés dans le sol. Certains signes portent à croire que des nématodes jouent un rôle dans la transmission des virus du sol.

Il est important de pouvoir déterminer aisément la présence d'un virus. L'application des méthodes existantes et les perspectives de perfectionnement et de développement de ces méthodes sont exposées en grandes lignes, en même temps que quelques-unes des méthodes nouvelles, qui ont été mises au point récemment et dont l'usage n'est pas encore généralisé.

La lutte contre les virus de la pomme de terre par des méthodes directes, c'est-à-dire par élimination du virus déjà présent dans la plante ou le tubercule au moyen d'un traitement par la chaleur et/ou par culture de méristèmes, est possible dans certains cas. Jusqu'ici, cependant, l'application de ces méthodes s'est montrée uniquement d'usage dans les cas où une variété de pomme de terre est totalement infectée par un virus et où elles servent donc à débarrasser du virus une variété entière.

Les mesures indirectes restent le principal mode de lutte contre les virus de la pomme de terre. L'auteur décrit les mesures à prendre pour obtenir des plants sains. Parmi ces mesures, citons l'arrachage précoce des plantes atteintes, la récolte précoce, l'emploi de tubercules prégermés et toutes les mesures pouvant accélérer l'obtention par la plante d'une résistance égale à celle de la plante mûre. L'usage d'insecticides systémiques pour détruire les pucerons vecteurs et la création de variétés immunes ou résistantes sont considérés comme des moyens importants de lutte contre les virus de la pomme de terre.

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